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Reversal of rivaroxaban-induced anticoagulation with prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant activated factor VII *in vitro* ☆☆

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ABSTRACT

Introduction: Anticoagulation therapies carry a risk of bleeding; reversal agents may be beneficial in cases of severe bleeding even for anticoagulants with a relatively short half-life, such as the oral factor Xa inhibitor rivaroxaban.
Materials and Methods: We investigated the *in vitro* reversal effect of prothrombin complex concentrate (PCC; 0.2-1.0 U/mL), activated PCC (aPCC; 0.2–1.0 U/mL) and recombinant activated factor VII (rFVIIa; 5–50 μg/mL) on rivaroxaban-induced (200–1000 ng/mL) changes in prothrombin time (PT) and thrombin generation (TG) in plasma, and in thromboelastometry (clotting time [CT]) in whole blood from healthy subjects.
Results: All three agents were partially effective in reversing rivaroxaban-induced anticoagulation but showed different profiles. rFVIIa and aPCC were more effective than PCC in reversing prolongations of PT, CT and TG lag time; rFVIIa was more effective than aPCC. However, the reversal effect reached a plateau with a maximal effect of approximately 50%. Inhibition of maximum thrombin concentration was slightly reversed by these agents; aPCC was the most effective. In contrast, inhibition of endogenous thrombin potential (ETP) was strongly reversed by aPCC, with significant increases over baseline at low rivaroxaban concentrations. Compared with aPCC, PCC showed a similar but less effective reversal profile. rFVIIa reversed ETP inhibition by approximately 50%.
Conclusions: The extent of reversal by aPCC, PCC and rFVIIa was dependent on the parameter measured in rivaroxaban-anticoagulated plasma or blood. ETP measurements may have predictive power for assessing the reversal potential of PCC or aPCC and may be used to indicate an increased prothrombotic risk.

Introduction

The direct oral anticoagulants, which target a single coagulation factor such as factor Xa (e.g. apixaban, edoxaban, rivaroxaban) or thrombin (e.g. dabigatran), have been developed to overcome some of the limitations of traditional anticoagulants. These limitations include parenteral administration with unfractionated heparin (UFH), low molecular weight heparins (LMWHs) and fondaparinux; the risk of heparin-induced thrombocytopenia with UFH (and, to a lesser extent, with LMWH); and the narrow therapeutic window, slow onset and offset of action, variable patient response, need for strict coagulation monitoring and numerous food and drug interactions with vitamin K antagonists (VKAs) [1–3]. Rivaroxaban, an oral, direct factor Xa inhibitor, has been assessed in large-scale phase III clinical trials across a broad spectrum of venous and arterial thromboembolic disorders [4–11]. In these trials, rivaroxaban was associated with non-inferior or superior efficacy and with a similar safety profile to the current standards of care (such as enoxaparin or warfarin). Based on these results, rivaroxaban is now licensed and used in clinical practice for the prevention and treatment of several thromboembolic disorders in adult patients.

All currently available anticoagulants carry a risk of bleeding. The effects of some traditional anticoagulants can be reversed, for example, protamine sulphate reverses the effect of UFH and partially neutralises LMWH, and vitamin K reverses the effect of VKAs. However, intravenous administration of vitamin K achieves normal

Abbreviations: ACCP, American College of Chest Physicians; ANOVA, analysis of variance; aPCC, activated prothrombin complex concentrate; CAT, calibrated automated thrombogram; C_{max}, maximum concentration (of thrombin); CT, clotting time; DMSO, dimethyl sulphoxide; ETP, endogenous thrombin potential; INR, international normalised ratio; LMWH, low molecular weight heparin; PCC, prothrombin complex concentrate; PPP, platelet-poor plasma; PT, prothrombin time; rFVIIa, recombinant activated factor VII; SEM, standard error of the mean; TEM, thromboelastometry; TF, tissue factor; TG, thrombin generation; UFH, unfractionated heparin; VKA, vitamin K antagonist.

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international normalised ratio (INR) levels only within 24 hours [3]. In patients with VKA-associated major bleeding events, the American College of Chest Physicians (ACCP) guidelines recommend the administration of four-factor prothrombin complex concentrate (PCC) in addition to vitamin K to replace clotting factors [12]. Currently, no clinically approved reversal agent exists for any of the direct oral anticoagulants. Of note, reversal agents would only be considered for the management of severe, life-threatening bleeding events. Current strategies for managing non-life threatening bleeding events include discontinuation of anticoagulation, delay of the next dose or the use of activated charcoal to reduce drug absorption [13–15]. However, anticoagulants with short half-lives are less likely to require a reversal agent than those with long half-lives, because withdrawal of the anticoagulant will normalise haemostasis. Warfarin reaches its peak anticoagulant effect between 72 and 96 hours after the initiation of therapy and has an effective half-life of between 20 and 60 hours with a mean of approximately 40 hours [3,16]. Because of the influence of warfarin on vitamin K-dependent clotting factors, its pharmacodynamic effects are more prolonged than its pharmacokinetic effects. In comparison, rivaroxaban has a short half-life (5–9 hours in younger subjects; 11–13 hours in elderly subjects aged 60–76 years) [17,18]. Furthermore, compared with warfarin, rivaroxaban was also associated with an improved bleeding profile in three phase III studies [8–10].

The ability to immediately reverse the anticoagulant effect of rivaroxaban may be needed in emergency situations, such as in patients with a life-threatening bleeding event or in case of urgent surgery. Recombinant activated factor VII (rFVIIa), activated prothrombin complex concentrate (aPCC) and PCC are haemostatic agents that contain a high concentration of coagulation factors and, therefore, may overcome factor Xa inhibition by enhancing thrombin generation (TG). rFVIIa and aPCC are approved to stop bleeding in patients with inhibitor-developing haemophilia [19–23]. PCC is approved and recommended for rapid warfarin reversal in patients with serious bleeding events [12,24,25]. It was anticipated that these agents may reverse the anticoagulation effect of rivaroxaban. Previous studies in animals and humans have demonstrated the potential of these agents in reversing rivaroxaban-induced anticoagulation [26–28].

The aim of this study was to assess the potential of PCC, aPCC and rFVIIa for the reversal of the anticoagulant activity of rivaroxaban in human blood in an *in vitro* system. The effects of these haemostatic agents on different parameters in terms of the reversal of rivaroxaban-induced anticoagulation were compared; parameters included prothrombin time (PT), clotting time (CT) measured by thromboelastometry (TEM) and parameters of TG (including lag time, maximum concentration of thrombin [C_{max}], and endogenous thrombin potential [ETP]).

Materials and Methods

Reagents

Rivaroxaban was prepared by Bayer Pharma AG (Wuppertal, Germany). Human rFVIIa (NovoSeven®) was supplied by Novo Nordisk (Copenhagen, Denmark). Nanofiltered aPCC (FEIBA NF®) was obtained from Baxter Germany (Unterschleiβheim, Germany), and four-factor PCC (Beriplex P/N®) was supplied by CSL Behring (Marburg, Germany). Rivaroxaban was dissolved in 100% dimethyl sulphoxide (DMSO) to achieve a stock solution of 1000 μg/mL and was further diluted in 100% DMSO to obtain plasma concentrations as used in PT and TEM; a stock solution of 500 μg/mL rivaroxaban was diluted further in 25% DMSO to obtain plasma concentrations as used in the TG assay. Adding rivaroxaban solutions to plasma resulted in DMSO concentration ≤ 1% (1% in the PT and TEM assays; 0.7–0.8% in the TG assay); these DMSO concentrations did not affect the assays. aPCC and PCC were reconstituted in demineralised water to obtain stock solutions of 100 U/mL; rFVIIa was reconstituted in demineralised water to obtain a stock solution of 1000 μg/mL; these stock solutions were further diluted in 0.9% saline to be used in assays. When calculating plasma or blood concentrations, a body weight of 70 kg with a blood volume of 5 L was assumed. The concentrations of rivaroxaban were chosen to simulate maximum plasma concentrations in patients treated with 20 mg once daily (creatinine clearance > 50 mL/min): mean 274 ng/mL (range 175–400 ng/mL) [29] and hypothetical overdoses (up to 1000 ng/mL in plasma and 1800 ng/mL in whole blood). aPCC concentrations were chosen to simulate human plasma concentrations of 0.7 U/mL (as expected after taking the recommended dose of 50 U/kg body weight); a higher concentration of 1.0 U/mL (recommended dose range 50–100 U/kg in patients with haemophilia A or B) [30] and the lower concentrations of 0.2 U/mL and 0.4 U/mL, were also investigated. The concentrations of PCC were chosen to simulate human plasma concentrations of 0.4 U/mL (25 U/kg body weight) and 0.7 U/mL (50 U/kg body weight) – recommended doses for patients treated with VKAs (INR 2.0–3.9 or INR > 6.0) [24] – and a lower concentration of 0.2 U/mL and higher concentration of 1.0 U/mL were also investigated. The lowest concentration of 5 μg/mL rFVIIa was chosen to simulate human plasma concentrations equivalent to 270 μg/kg (recommended dose 90 μg/kg every 2 hours) [31], and higher concentrations of 15 μg/mL and 50 μg/mL (corresponding to a concentration 30-times higher than the C_{max} of the approved 90 μg/kg rFVIIa) were also investigated.

Collection and Preparation of Blood Samples

Individual blood was collected by venipuncture from healthy subjects who had not received medication in the previous 10 days. Blood was collected into vacutainer tubes containing 1/10 volume of 3.12% trisodium citrate (Sarstedt, Nümbrecht, Germany). Platelet-poor plasma (PPP) from individual subjects was obtained by immediate centrifugation at 1000 *g* for 20 minutes at room temperature and was stored in aliquots at −70 °C. PPP of six different subjects was thawed and pooled directly before measurements were taken (PT and TG assays). In whole blood, haematocrit was measured to calculate the individual plasma concentrations of rivaroxaban.

Prothrombin Time

PT was measured in a coagulometer (Amelung KC 10A, Lemgo, Germany). Samples were prepared as follows: pooled PPP was spiked with rivaroxaban (200, 500 or 1000 ng/mL) in the presence or absence of aPCC (0.2, 0.4, 0.7 or 1.0 U/mL; n = 5), PCC (0.2, 0.4, 0.7 or 1.0 U/mL; n = 7) or rFVIIa (5, 15 or 50 μg/mL; n = 7). Samples (duplicates) were incubated for 3 minutes at 37 °C and the reaction was started by the addition of Néoplastine Plus® (Diagnostica Stago, Asnières-sur-Seine, France) in accordance with the manufacturer's instructions. Control curves were generated with the appropriate vehicles (n = 5–12).

Thromboelastometry

CT was determined by TEM using tissue factor (TF) to initiate coagulation (*r* ex-tem®; TEM Innovations GmbH, Munich, Germany) in accordance with the manufacturer's instructions. Freshly drawn whole blood samples (duplicates) were prepared as follows: whole blood was spiked with rivaroxaban (200, 500 or 1000 ng/mL) in the presence or absence of aPCC (0.2, 0.7 or 1.0 U/mL; n = 5), PCC (0.2, 0.4 or 1.0 U/mL; n = 2–3) or rFVIIa (5, 15 or 50 μg/mL; n = 4). The blood was stored at room temperature on a rotating mixer and processed within 15–90 minutes after venipuncture. The reaction was started by adding *r* ex-tem (thromboplastin from rabbit brain) and $CaCl_2$ (star-tem reagent, $CaCl_2$ 11.8 mM; final concentration) to the samples in a ROTEM analyser (TEM Innovations GmbH, Munich,

Germany). Run time was 60 minutes. Control curves were generated with the appropriate vehicles (n = 7–15). The plasma concentrations of rivaroxaban and the haemostatic agents were calculated for each sample. Plasma concentrations are given as mean values obtained from the individual donors.

Thrombin Generation Assay

TG was measured using the calibrated automated thrombogram (CAT; Diagnostica Stago, France) method in accordance with the manufacturer's instructions using a fluorometer (Fluoroskan Ascent; Thermo-Labsystems, Germany). Samples (in triplicate) were prepared as follows: pooled PPP was spiked with rivaroxaban (200, 500 or 1000 ng/mL) in the presence or absence of aPCC (0.2, 0.4, 0.7 or 1.0 U/mL; n = 5), PCC (0.2, 0.4, 0.7 or 1.0 U/mL; n = 7) or rFVIIa (5, 15 or 50 μg/mL; n = 6–7). PPP reagent (5 pM; Diagnostica Stago, France) was added to the samples before the reaction was started by the addition of FluCa (HEPES pH 7.5 containing $CaCl_2$ and fluorogenic substrate Z-Gly-Gly-Arg-AMC [Bachem, Switzerland]). TG was assessed by measuring the cleavage of the fluorogenic substrate. Lag time, C_{max} and ETP were determined. Control curves were generated with the appropriate vehicles (n = 5–8). ETP could not be calculated at the highest concentrations of aPCC and PCC in rivaroxaban-free plasma, because the amount of thrombin generated under these conditions exceeded the upper detection limit of the assay.

Statistical Methods

Analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used for statistical analysis (Graph Pad Prism version 5.04; Graph Pad Software Inc., San Diego, CA, USA). Statistical comparisons were made between rivaroxaban and rivaroxaban plus the haemostatic agent at each rivaroxaban concentration, as well as between unspiked control and all spiked samples; p-values < 0.05 were considered to be statistically significant. Results are shown as means ± standard error of mean (SEM).

Results

Prothrombin Time

Rivaroxaban at 200–1000 ng/mL concentration-dependently prolonged PT in human plasma by 2.1-fold to 5.5-fold over baseline.

rFVIIa alone at concentrations of between 5 μg/mL and 50 μg/mL significantly shortened PT from 12.9 seconds to a minimum of 7.3 seconds (up to 0.58-fold) (Fig. 1A). All concentrations of rFVIIa significantly reversed rivaroxaban-induced PT prolongation in plasma: by 47–48%, 53–55% and 53–54% with 200, 500 and 1000 ng/mL rivaroxaban, respectively (Fig. 1A). Because maximal reversal was already achieved by 5 μg/mL rFVIIa at all concentrations of rivaroxaban, the concentration dependency of rFVIIa was further investigated for concentrations between 0.001 μg/mL and 5 μg/mL. rFVIIa at 0.005 μg/mL was the minimum effective concentration for the reversal of 1000 ng/mL rivaroxaban in the PT assay (data not shown). Higher concentrations of rFVIIa reversed rivaroxaban-induced PT prolongation in a concentration-dependent manner up to 5 μg/mL.

aPCC alone at concentrations between 0.2 U/mL and 1.0 U/mL shortened PT in a concentration-dependent manner from 12.8 seconds to 9.2 seconds (up to 0.71-fold) (Fig. 1B). PT prolongation in plasma containing 200, 500 and 1000 ng/mL rivaroxaban was significantly reversed by 22–38%, 32–49% and 30–47%, respectively, across all concentrations of aPCC (Fig. 1B). With aPCC, a ceiling effect of reversal of rivaroxaban-induced PT prolongation was achieved at 0.7 U/mL (Fig. 1B). PCC alone at concentrations between 0.2 U/mL and 1.0 U/mL slightly shortened PT from 13.1 seconds to 12.3 seconds (up to 0.94-fold) (Fig. 1C). PCC significantly reversed PT prolongation by 15–22% in plasma containing 500 and 1000 ng/mL rivaroxaban. In the presence of 200 ng/mL rivaroxaban, the reversal effect (11–16%) did not reach statistical significance (Fig. 1C). In summary, the reversal effect decreased in the following order rFVIIa > aPCC > PCC (Table 1).

Thromboelastometry

In the whole blood coagulation assay, rivaroxaban plasma concentrations between 331 ng/mL and 1807 ng/mL concentration-dependently prolonged CT from 1.8-fold to 3.7-fold over baseline (Fig. 2).

With rFVIIa alone (9, 27 and 87 μg/mL), CT was reduced up to 0.67-fold from 98 seconds to 66, 82 and 70 seconds, respectively (Fig. 2A). Maximum reversal of CT prolongation with 358, 895 and 1807 ng/mL rivaroxaban by 37–53% was achieved at the lowest concentration of 9 μg/mL rFVIIa. aPCC alone at 0.33, 1.16 and 1.64 U/mL shortened CT up to 0.7-fold from 93 seconds to 65, 73 and 73 seconds, respectively (Fig. 2B). CT prolongation with 331, 827 and 1656 ng/mL rivaroxaban was reversed by 9–25% with 0.33 U/mL and 1.16 U/mL aPCC, and by 24–40% with 1.64 U/mL aPCC, but this effect only reached statistical significance for 1656 ng/mL rivaroxaban. These results indicated that aPCC partially reversed rivaroxaban-induced prolongation of CT but not in a concentration-dependent manner and was less effective than rFVIIa (Table 1). PCC alone (0.35, 0.67 and 1.68 U/mL) did not affect CT and did not reverse rivaroxaban-induced CT prolongation (Table 1, Fig. 2C).

Thrombin Generation

As expected [32], rivaroxaban affected all parameters of TG in a concentration-dependent manner. Lag time was prolonged by between 3.0-fold and 4.9-fold relative to baseline, at rivaroxaban concentrations of 200, 500 and 1000 ng/mL (Fig. 3). ETP was inhibited by between 28% and 84% (Fig. 4), and C_{max} was inhibited by between 86% and 97% relative to baseline at rivaroxaban concentrations of 200, 500 and 1000 ng/mL (Fig. 5).

rFVIIa alone shortened lag time by 0.6-fold relative to baseline (Fig. 3A), slightly but significantly increased C_{max} by 9–10% (Fig. 5A) but had no effect on ETP (Fig. 4A). All concentrations of rFVIIa significantly reversed rivaroxaban-induced prolongation of lag time by 50–57% at 200, 500 and 1000 ng/mL rivaroxaban (Fig. 3A). All concentrations of rFVIIa significantly reversed rivaroxaban-induced ETP inhibition by 39–56% (Fig. 4A) and C_{max} inhibition by 6–18% (although not all concentrations reached statistical significance; Fig. 5A) at 200, 500 and 1000 ng/mL rivaroxaban.

aPCC alone slightly shortened lag time at all concentrations by 20–30% relative to baseline (Fig. 3B), and increased ETP in a concentration-dependent manner by 29%, 56% and 99% at 0.2, 0.4 and 0.7 U/mL, respectively (Fig. 4B). In addition, aPCC alone increased C_{max} concentration dependently by 32%, 57%, 83% and 122% over baseline at aPCC concentrations of 0.2, 0.4, 0.7 and 1.0 U/mL, respectively (Fig. 5B). All concentrations of aPCC reversed rivaroxaban-induced prolongation of lag time by 33–47% (Fig. 3B). In plasma spiked with 200 ng/mL rivaroxaban, ETP was significantly increased by 14%, 58% and 81% over baseline at aPCC concentrations of 0.4, 0.7 and 1.0 U/mL, respectively (Fig. 4B). In plasma spiked with 500 ng/mL rivaroxaban, reversal of rivaroxaban-induced ETP inhibition was not significant with 0.2 U/mL aPCC but was significant with 0.4 U/mL aPCC (89%), whereas ETP increased by 27% and 32% over baseline with 0.7 U/mL ($p < 0.001$) and 1.0 U/mL aPCC, respectively (Fig. 4B). At a concentration of 1000 ng/mL rivaroxaban, ETP inhibition was reversed by 40–73% at all concentrations of aPCC ($p < 0.01$ for concentrations ≥ 0.4 U/mL; Fig. 4B). aPCC (all tested concentrations) reversed rivaroxaban-induced inhibition of C_{max} by 20–56%, 12–26% and 6–13% at 200, 500 and 1000 ng/mL rivaroxaban, respectively, although not all values reached statistical significance (Fig. 5B).

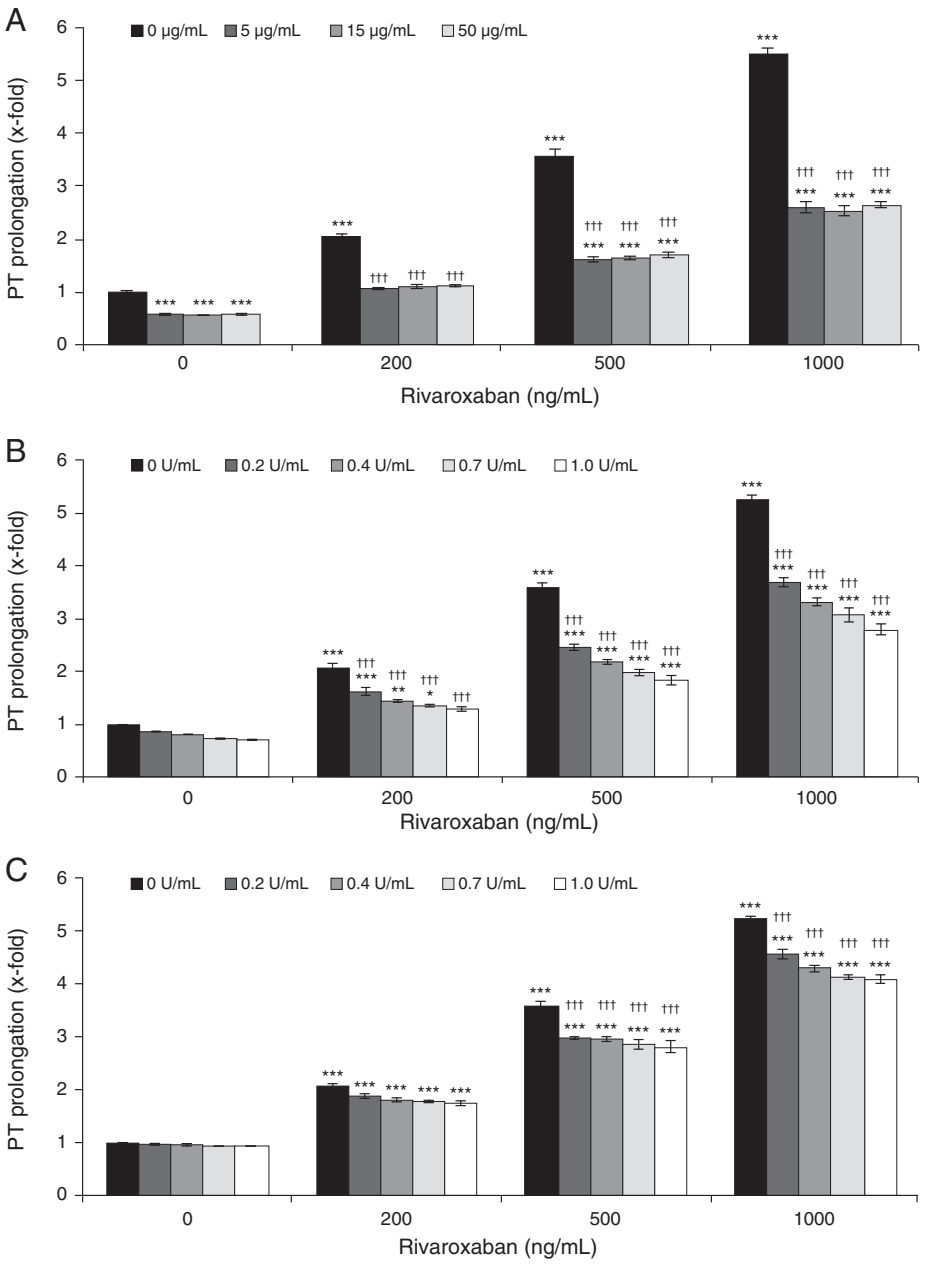

Fig. 1. Reversal of rivaroxaban-induced prothrombin time (PT) prolongation by increasing concentrations of (A) recombinant activated factor VII (0, 5, 15, 50 µg/mL; n = 7), (B) activated prothrombin complex concentrate (0, 0.2, 0.4, 0.7, 1.0 U/mL; n = 5), and (C) prothrombin complex concentrate (0, 0.2, 0.4, 0.7, 1.0 U/mL; n = 7) in human plasma. Rivaroxaban concentrations were 0, 200, 500 and 1000 ng/mL. Values are shown as mean x-fold change over baseline ± standard error of the mean. Baseline values were measured in the absence of rivaroxaban and the reversal agent. *p < 0.05 vs. control, **p < 0.01 vs. control, ***p < 0.001 vs. control, †††p < 0.001 vs. rivaroxaban.

PCC alone had no significant effect on lag time (Fig. 3C), but it concentration-dependently increased ETP by 37–62% (at concentrations of 0.2–0.7 U/mL; Fig. 4C) and C_{max} by 19–67% (at concentrations of 0.2–1.0 U/mL; Fig. 5C). All concentrations of PCC had a small – and in most cases significant – reversal effect of 5–15% on rivaroxaban-induced prolongation of lag time (Fig. 3C). In plasma spiked with 200 ng/mL rivaroxaban, ETP was significantly reversed by 70% with 0.2 U/mL PCC; at higher PCC concentrations of 0.4, 0.7 and 1.0 U/mL, ETP increased above baseline and reached reversal levels of 105%, 118% and 143%, respectively (Fig. 4C). In plasma spiked with 500 and 1000 ng/mL rivaroxaban, ETP was reversed by 30–48% and 12–21%, respectively, at all concentrations of PCC (Fig. 4C). PCC reversed the inhibition of C_{max} in plasma spiked with 200 ng/mL rivaroxaban by 8–14% (not significant) and had no reversal effect in plasma spiked with 500 ng/mL or 1000 ng/mL rivaroxaban (Fig. 5C).

In summary, lag time of TG was partially reversed by all three agents; rFVIIa had the greatest reversal effect followed by aPCC, and PCC had the least effect. Whether ETP inhibition was completely reversed or even overcompensated by aPCC or PCC was dependent on the rivaroxaban concentration used to induce inhibition; aPCC was more effective than PCC. rFVIIa showed a strong but only partial reversal. rFVIIa and aPCC partially reversed rivaroxaban-induced inhibition of C_{max}; PCC had no effect (Table 1).

Discussion

Owing to the lack of predictive assays for the haemostatic efficacy of non-specific reversal agents, the studies presented in this article investigated the reversal potential of aPCC (FEIBA NF), PCC (Beriplex P/N) and rFVIIa (NovoSeven) as measured by different clot-based assays

Table 1
Comparison of reversal effects of rFVIIa, aPCC and PCC on rivaroxaban-induced changes in PT and parameters (lag time, ETP, C_{max}) of TG in plasma and CT (thrombelastometry) in whole blood compared with the rivaroxaban effect in the absence of the reversal agent (% reversal ± SEM). For comparison of the effectiveness, the lowest and the highest concentration of rFVIIa, aPCC and PCC tested were chosen and the clinical relevant concentrations for rivaroxaban.

Parameter	Rivaroxaban (ng/mL)	rFVIIa (μg/mL)		aPCC (U/mL)		PCC (U/mL)	
		5	50	0.2	1.0	0.2	1.0
PT prolongation	200	48 ± 1	47 ± 1	22 ± 3	38 ± 2	11 ± 4	16 ± 3
	500	55 ± 1	53 ± 1	32 ± 1	49 ± 3	19 ± 3	22 ± 1
TG lag time prolongation	200	57 ± 1	57 ± 1	33 ± 3	47 ± 3	6 ± 6	13 ± 3
	500	53 ± 1	55 ± 1	35 ± 5	43 ± 5	5 ± 3	15 ± 3
TG ETP inhibition	200	43 ± 4	39 ± 4	102 ± 2[b]	181 ± 10[b]	70 ± 10	143 ± 8[b]
	500	53 ± 2	56 ± 2	75 ± 11	132 ± 10[b]	30 ± 4	48 ± 9
TG C_{max} inhibition	200	14 ± 1	18 ± 1	20 ± 3	56 ± 8	8 ± 0.4	14 ± 1.1
	500	8 ± 1	12 ± 1	12 ± 3	26 ± 5	2 ± 0.2	3 ± 1.1
CT prolongation[a]	200	37 ± 7	36 ± 10	23 ± 7	28 ± 10	−9 ± 4	−12 ± 4
	500	41 ± 3	37 ± 4	13 ± 7	24 ± 6	−9 ± 5	−7 ± 7

Categorisation of strength of reversal was assessed by mean value of % reversal values per parameter.

No reversal	Low reversal	Medium reversal	Strong reversal	Nearly complete
mean 0%	mean 15%	mean 36%	mean 51%	or reversal >100%

[a]Concentration of rivaroxaban and reversal agent are given as whole blood concentrations. In plasma, the appropriate concentrations were 331–358 ng/mL and 827–895 ng/mL rivaroxaban, 9 and 87 μg/mL rFVIIa, 0.33 and 1.64 U/mL aPCC, 0.35 and 1.68 U/mL PCC.
[b]Calculated as increase of ETP over control value (%).
Abbreviations: aPCC, activated prothrombin complex concentrate; C_{max}, maximum thrombin concentration; CT, clotting time; ETP, endogenous thrombin potential; PCC, prothrombin complex concentrate; PT, prothrombin time; rFVIIa, recombinant activated factor VII; SEM, standard error of the mean; TG, thrombin generation.

(PT, CT) and TG tests in rivaroxaban-anticoagulated human whole blood or PPP. Our goal was to assess the effects of potential reversal strategies and to provide data on the strengths and limitations of these laboratory assays.

It is important to note that different formulations of PCC are available commercially. These differ regarding the inclusion of coagulation inhibitors (heparin, protein C, protein S, protein Z and antithrombin) and in their content of coagulation factors (some contain limited amounts of factor VII or differing amounts of factor II, VII and X in relation to factor IX), which may lead to different results [33]. This study tested the reversal potential of the four-factor PCC Beriplex P/N.

The results showed that rivaroxaban-induced prolongation of PT (measured using Néoplastine®), CT and lag time of TG, and reduction of C_{max} and ETP of TG were in agreement with previously reported data [32,34,35] and confirmed that these assays are useful tools for measuring the effects of factor Xa inhibition by rivaroxaban. Therefore, these assays were used to estimate the reversal potential of non-specific haemostatic agents.

All three haemostatic agents were partially effective in reversing rivaroxaban-induced anticoagulation but showed different profiles. Both factor VIIa-containing agents, rFVIIa and aPCC, were more effective than PCC in reversing the kinetic parameter of the initiation phase of coagulation measured by PT, CT and lag time of TG. rFVIIa was more effective than aPCC in the clot-based assays. However, even at the highest concentration, rFVIIa and aPCC did not reduce clot times to baseline; the reversal of PT, CT and lag time of TG reached a plateau at approximately 50% or lower. In contrast, PCC only slightly reversed rivaroxaban-induced prolongation of PT and lag time of TG and did not affect CT. Both rFVIIa and aPCC alone shortened PT, CT and lag time of TG, but PCC alone only slightly shortened PT, suggesting that the reversal effect on clot times may at least be partly owing to the effect of the agent itself on the measurement by accelerating the initiation phase of coagulation. Furthermore, during the initiation phase, the amount of factor Xa generated is not sufficient to overcome inhibition by rivaroxaban and to neutralise the anticoagulant effect of rivaroxaban. A previous study showed that when assessing concentrations of coagulation factors generated during the initiation phase, only a small amount of thrombin (26 ± 6.2 nM) and prothrombinase (1.3 pM) is generated in a whole blood coagulation assay [36]. The plasma concentrations of rivaroxaban used in our study were in the range 460–4100 nM (MW 436 g). Therefore, clot-based assays may not precisely predict the reversal potential of haemostatic agents because they measure the initiation phase of coagulation and do not reflect the whole amount of factor Xa generated during the coagulation process. Recently, similar results were obtained by Körber et al. in an *in vitro* study demonstrating that PCC caused no significant reversal in PT and CT in human plasma spiked with rivaroxaban [37]. rFVIIa significantly reversed rivaroxaban-induced prolongation of PT and CT [37]. Dinkelaar et al., also demonstrated that PCC did not result in normalisation of PT and TG lag time [38]. Furthermore, similar results were obtained with the factor Xa inhibitor edoxaban, demonstrating the largest reversal effect on PT prolongation by FVIIa, followed by aPCC and the smallest effect by PCC *in vitro* in human plasma [39]. However, in contrast to our results Escolar et al. recently showed that prolongation of CT by apixaban (ROTEM) was completely reversed or even overcompensated by rFVIIa and aPCC; the reversal effect with PCC was less pronounced [40]. These differences may be explained by the use of a lower $CaCl_2$ concentration for recalcification (6 mM) in Escolar et al. [40].

The potential of PCC to reverse the anticoagulant effect of rivaroxaban has also been investigated previously in healthy human subjects [26]. However, in this study, PCC 50 U/kg rapidly and completely reversed the prolongation of PT induced by 20 mg twice-daily rivaroxaban [26]. The prolongation of PT with rivaroxaban was only 1.3-fold, indicating that the PT reagent used (Siemens Healthcare Diagnostics, Marburg, Germany) was less sensitive to rivaroxaban; therefore, the observed complete reversal by PCC may have been as a result of the PT reagent used rather than a 'true' reversal [26]. PT prolongation by rivaroxaban

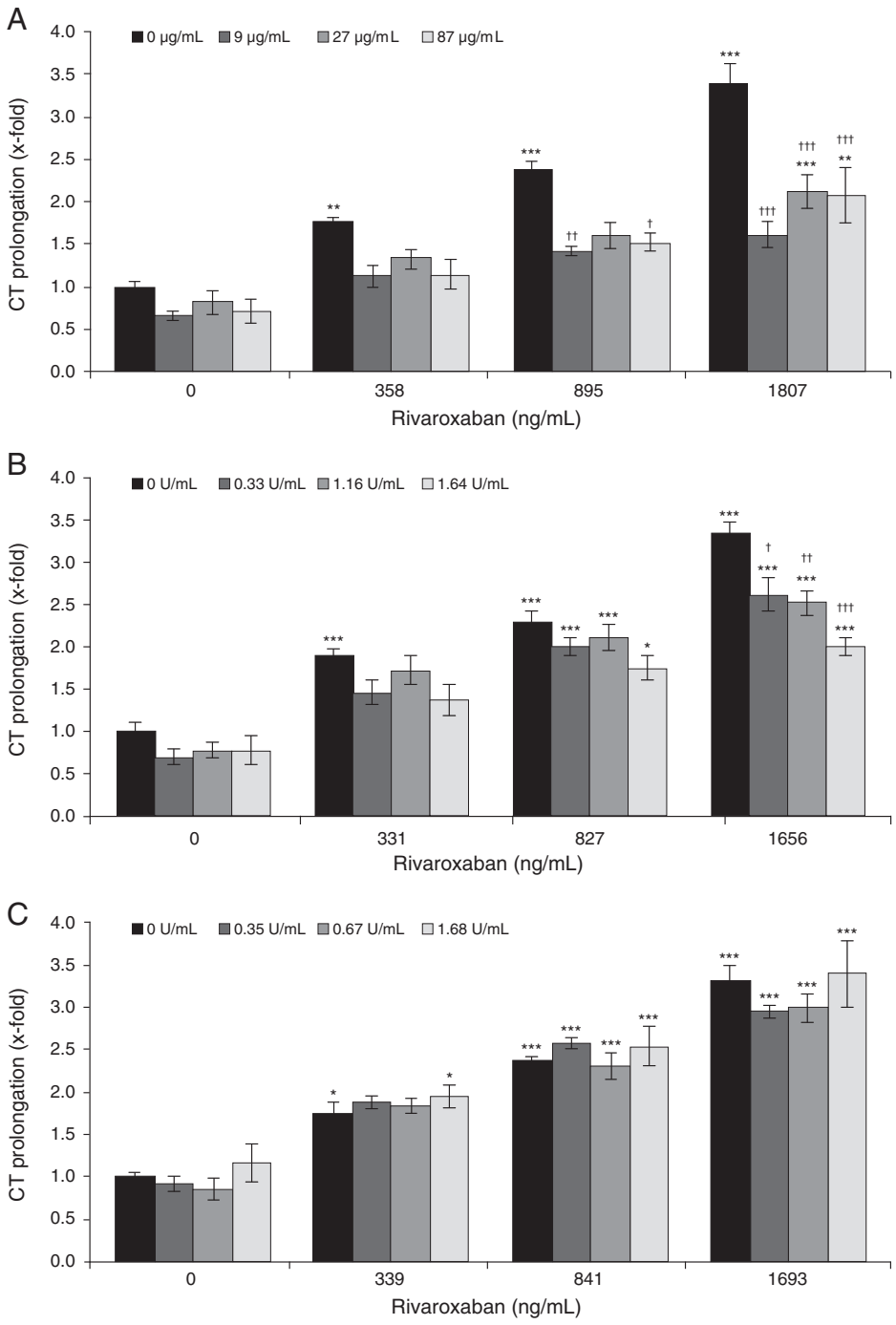

Fig. 2. Reversal of rivaroxaban-induced clotting time (CT) prolongation of the extrinsic coagulation pathway (*r*-extem reagent-induced and measured using thromboelastometry) by (A) recombinant activated factor VII (0, 9 ± 0.9, 27 ± 0.9 and 87 ± 1.4 μg/mL; n = 4), (B) activated prothrombin complex concentrate (0, 0.33 ± 0.01, 1.16 ± 0.04, 1.64 ± 0.04 U/mL; n = 5), and (C) prothrombin complex concentrate (0, 0.35 ± 0.01, 0.67 ± 0.01, 1.68 ± 0.05 U/mL; n = 2–3) in human whole blood. Individual plasma concentrations were calculated for each individual donor by taking into account the individual haematocrit. All values are mean x-fold change over baseline ± standard error of the mean. Baseline values were measured in the absence of rivaroxaban and the reversal agent. *$p < 0.05$ vs. control, **$p < 0.01$ vs. control, ***$p < 0.001$ vs. control, †$p < 0.05$ vs. rivaroxaban, ††$p < 0.01$ vs. rivaroxaban, †††$p < 0.001$ vs. rivaroxaban.

varied depending on the reagent used [34], indicating that the extent of reversal measured may depend on the thromboplastin reagent used in the assay. In addition, it cannot be excluded that the differences in reversal, as reported by Eerenberg et al. and in our study, stem from different study designs, namely *in vivo* versus *in vitro*, respectively [26]. The *in vivo* administration of PCC could influence the final composition of PCC in plasma, and TF may have been exposed at the site of blood withdrawal and may have influenced the *ex vivo* measurements.

When measuring TG, measurement of ETP refers to the total amount of thrombin generated during the initiation, propagation and amplification phases of coagulation [35,41]. aPCC alone and PCC alone, but not rFVIIa alone, concentration-dependently increased the ETP over non-anticoagulated control values. Both aPCC and PCC concentration-dependently reversed ETP reduction induced by rivaroxaban. Complete neutralisation was obtained dependent on both the rivaroxaban concentration and the aPCC or PCC concentration. However, at clinically relevant concentrations

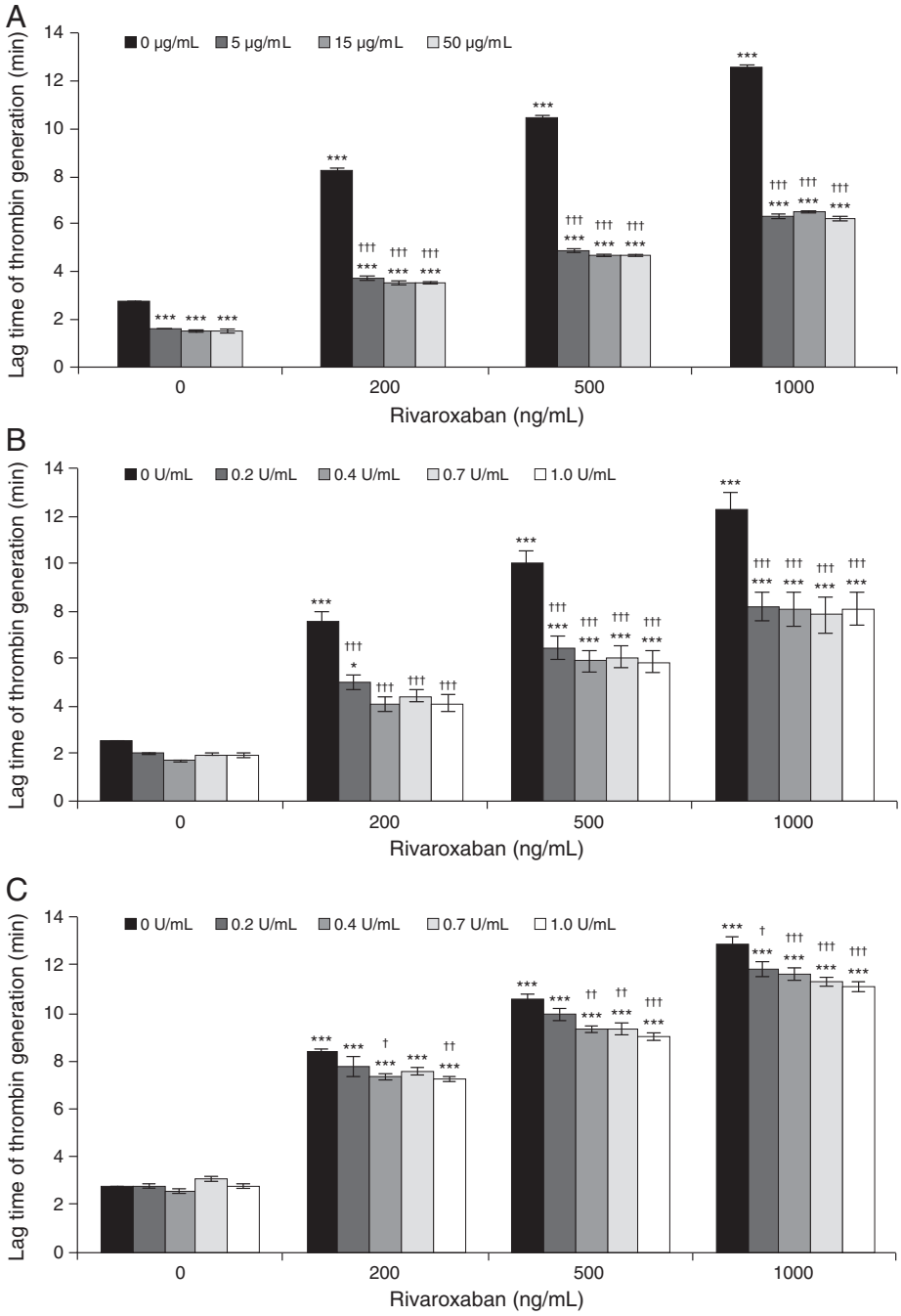

Fig. 3. Reversal of rivaroxaban-induced prolongation of lag time (min) of thrombin generation by (A) recombinant activated factor VII (0, 5, 15, 50 µg/mL; n = 6–8), (B) activated prothrombin complex concentrate (0, 0.2, 0.4, 0.7, 1.0 U/mL; n = 5), and (C) prothrombin complex concentrate (0, 0.2, 0.4, 0.7, 1.0 U/mL; n = 7) in human plasma. Values are shown as mean ± standard error of the mean. Baseline values were measured in the absence of rivaroxaban and the reversal agent. *p < 0.05 vs. control, ***p < 0.001 vs. control, †p < 0.05 vs. rivaroxaban, ††p < 0.01 vs. rivaroxaban, †††p < 0.001 vs. rivaroxaban.

of rivaroxaban, PCC (at 200 ng/mL) and aPCC (at 200 ng/mL and 500 ng/mL) increased ETP over the control value at 0.7 U/mL (corresponding to ~ 50 U/kg) and 1.0 U/mL. These data indicate that the haemostatic agents tested have the potential to reverse the anticoagulant effect of rivaroxaban and also show their prothrombotic potential. rFVIIa partially corrected the ETP with a maximal effect of 39–56% at all concentrations of rivaroxaban. This may be caused by enhancement of the TF pathway and the direct activation of factor IX by rFVIIa [42]. However, at the concentrations described in this study, rFVIIa did not reverse the thrombin generation capacity or the clot-based parameters (PT, CT and lag time of TG) in a concentration-dependent manner. This was in contrast to the reversal effects of aPCC and PCC. These results are in agreement with those described by Desmurs-Clavel et al. [43], a similar study that compared the reversal effects of rFVIIa, aPCC and PCC in fondaparinux-anticoagulated platelet rich plasma. Importantly, we observed a concentration-dependent reversal of rivaroxaban-induced PT prolongation at concentrations of ≤ 5 µg/ml rFVIIa with a minimum effective concentration of ~ 5 ng/mL, corresponding to ~ 0.27 µg/kg (data not shown).

Similar to our study, Dinkelaar et al. demonstrated that PCC normalised total thrombin potential in rivaroxaban-spiked plasma and whole blood *in vitro* [38]. Marlu et al. obtained similar results in an *ex vivo* study that compared the effects of PCC (Konokad), aPCC

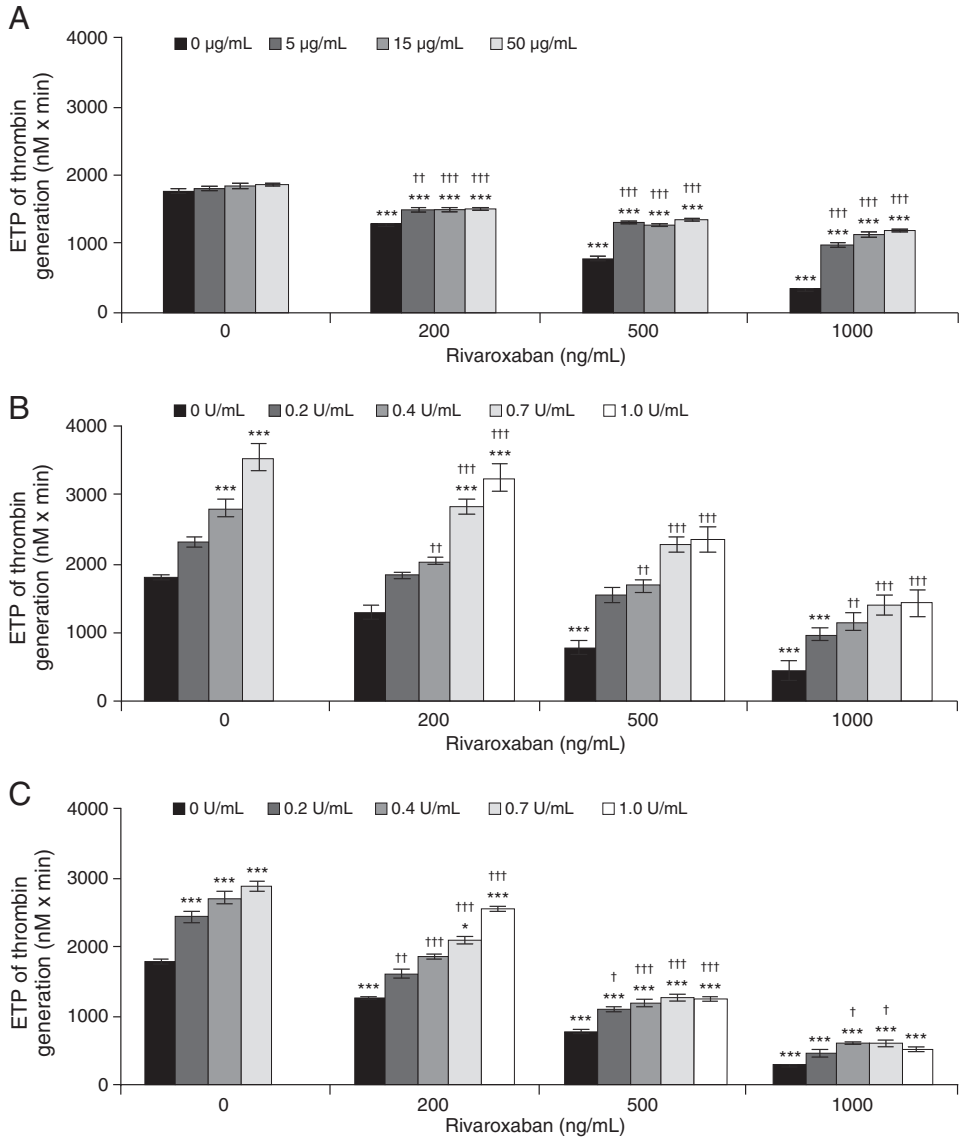

Fig. 4. Reversal of rivaroxaban-induced inhibition of the endogenous thrombin potential (ETP) of thrombin generation (nM × min) by (A) recombinant activated factor VII (0, 5, 15, 50 µg/mL; n = 6–8), (B) activated prothrombin complex concentrate (0, 0.2, 0.4, 0.7, 1.0 U/mL; n = 5), and (C) prothrombin complex concentrate (0, 0.2, 0.4, 0.7, 1.0 U/mL; n = 6). ETP could not be calculated at 1.0 U/mL activated prothrombin complex concentrate or prothrombin complex concentrate in rivaroxaban-free plasma, because the amount of thrombin generated under these conditions exceeded the upper detection limit of the assay. Values are shown as mean ± standard error of the mean. Baseline values were measured in the absence of rivaroxaban and the reversal agent. *p < 0.05 vs. control, **p < 0.01 vs. control, ***p < 0.001 vs. control, †p < 0.05 vs. rivaroxaban, ††p < 0.01 vs. rivaroxaban, †††p < 0.001 vs. rivaroxaban.

(FEIBA) and rFVIIa on the parameters of TG in healthy volunteers who had received 20 mg rivaroxaban [44]. In this study, PCC strongly reversed ETP, and aPCC reversed the kinetic parameters (lag time and time to maximum thrombin concentration) and ETP [44]. rFVIIa reversed the kinetic parameters, but in contrast to our study, rFVIIa had no effect on ETP despite the use of higher rFVIIa concentrations. Eerenberg et al. [26] measured the effect of PCC on the ETP in healthy human subjects treated with rivaroxaban and also demonstrated complete reversal of ETP. Complete reversal of ETP by aPCC was also demonstrated in human plasma spiked with the indirect factor Xa inhibitor fondaparinux [43].

Rivaroxaban-induced inhibition of C_{max} of TG was affected by the three agents, with aPCC and PCC alone significantly increasing C_{max} over baseline. However, the reversal effect was much less pronounced compared with ETP. Rivaroxaban alone potently reduced C_{max}, and to a lesser extent ETP, with IC_{50} values of 20 ng/mL and 458 ng/mL (mean of the three studies measuring the effects of PCC, aPCC and rFVIIa), respectively. These results suggest that the main effect of rivaroxaban-induced factor Xa inhibition is the delay of thrombin formation in the TG assay. Thus, in rivaroxaban-spiked plasma even in the presence of aPCC, PCC or rFVIIa, the generation of thrombin and, therefore, factor Xa is delayed, and the amount of factor Xa generated is still insufficient to neutralise rivaroxaban at the time point of C_{max}. Similarly, in the *ex vivo* study by Marlu et al. [44], the effect on C_{max} reversal was less pronounced compared with the effect on ETP.

In summary, the reversal of rivaroxaban-induced anticoagulation by rFVIIa and aPCC may be assessed using PT, CT, lag time and ETP, but not by C_{max}. Owing to the concentration dependency of the reversal effect needed to achieve maximal reversal demonstrated in this study, ETP measurements may be more useful in predicting the reversal potential of aPCC and PCC. In addition, measuring this parameter may also indicate the prothrombotic potential of the haemostatic agent.

There are also various issues regarding the transferability of the assays from the laboratory to daily clinical practice. Thromboelastometry is a simple, point-of-care assay that is available in many hospitals. However, the sensitivity of the currently available CT assays is considered insufficient and a more sensitive CT assay would be highly desirable. In contrast, TG assays are sensitive, but are not routinely available in the

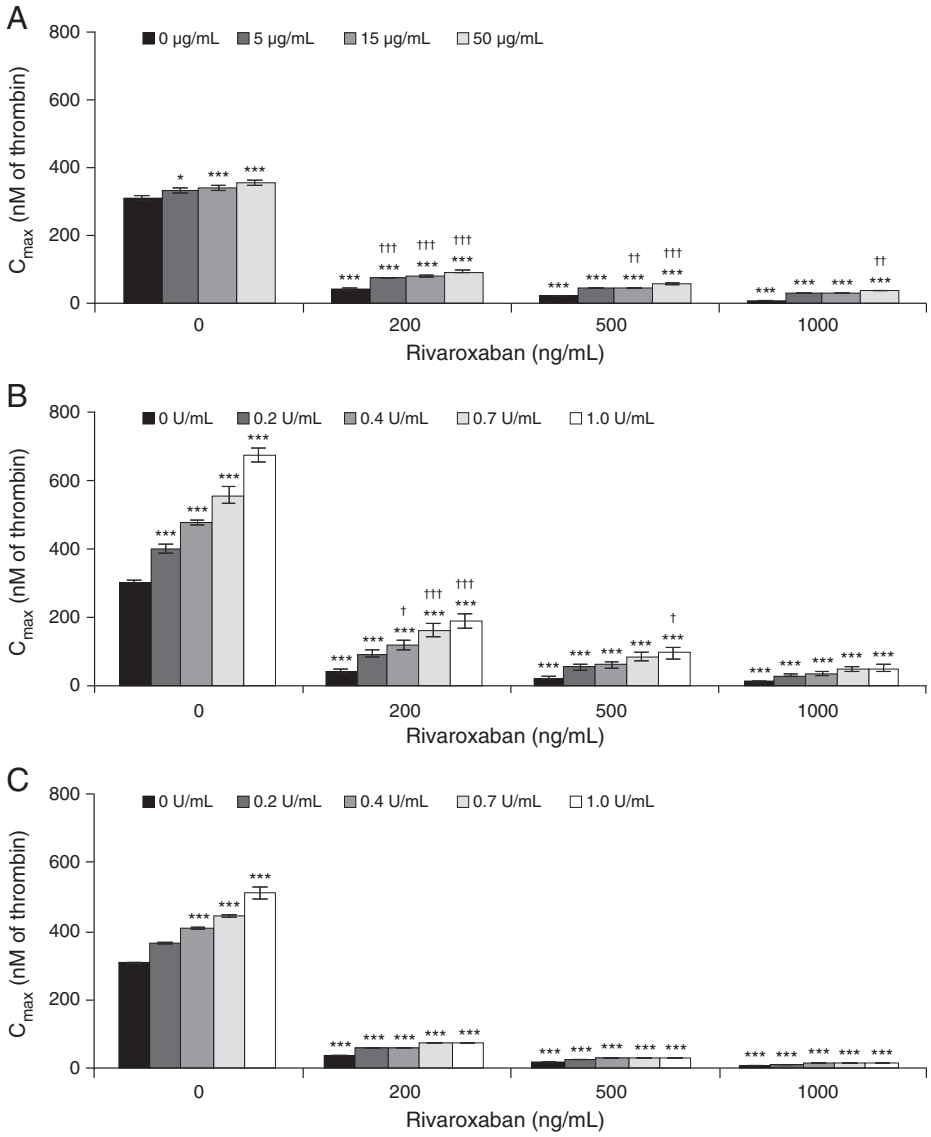

Fig. 5. Reversal of the rivaroxaban-induced reduction in maximum thrombin concentration (C_{max}; nM thrombin) by (A) recombinant activated factor VII (0, 5, 15, 50 µg/mL; n = 6–8), (B) activated prothrombin complex concentrate (0, 0.2, 0.4, 0.7, 1.0 U/mL; n = 5), and (C) prothrombin complex concentrate (0, 0.2, 0.4, 0.7, 1.0 U/mL; n = 7) in human plasma. Values are shown as mean ± standard error of the mean. Baseline values were measured in the absence of rivaroxaban and the reversal agent. $^{*}p < 0.05$ vs. control, $^{***}p < 0.001$ vs. control, $^{\dagger}p < 0.05$ vs. rivaroxaban, $^{\dagger\dagger}p < 0.01$ vs. rivaroxaban, $^{\dagger\dagger\dagger}p < 0.001$ vs. rivaroxaban.

clinical setting. Moreover, owing to the sophisticated nature of TG assays, results are not immediately available. In addition, there are some general limitations to be considered due to the nature of the assay. For example, the reversal potential of PCC as measured by TG assays was dependent on the concentration of TF in the assay [38]. Another study investigating the reversal of apixaban-induced changes in TG by rFVIIa, aPCC or PCC, also showed that the measured reversal effect was dependent on the activating reagents [40]. Computational modelling data from healthy subjects and patients showed that variations in TG did not mirror the variations observed for factor Xa generation, and individuals with similar TG profiles differed in their factor Xa generation profiles [45]. Importantly, we used plasma from healthy volunteers in our study, which is a limitation that needs to be considered when interpreting the results. In addition, the TG assay may not fully reflect the biology of clot formation in patients receiving anticoagulants.

A major limitation of all *in vitro* assays is that they do not mirror the complex situation of the individual patient with a bleeding event, including circulating blood with its cellular components and the damaged vessel. It is, therefore, important to note, that there is no experience of how the reversal effects measured by laboratory assays correlate with bleeding control or the reversal agents' prothrombotic potential in the clinical situation. We cannot exclude that the commercially available laboratory assays may have poor predictive value for the management of bleeding in patients treated with rivaroxaban. Therefore, clinical studies are necessary to investigate which assay or assay conditions best predict haemostasis in patients who are treated with rivaroxaban and a reversal agent (e.g. in emergency situations).

The potential of aPCC, PCC and rFVIIa to reverse the anticoagulant effect of rivaroxaban has also been investigated previously in animal models, including rats, mice and baboons. In these studies, aPCC, PCC and rFVIIa were shown to at least partially reverse the anticoagulant effect of rivaroxaban, including PT and bleeding time *ex vivo and in vivo* [27,28]. This was in contrast to a previous study in a rabbit hepatosplenic haemorrhagic model, which demonstrated that rFVIIa and PCC did not reduce rivaroxaban-induced bleeding [46]. However, it is noteworthy that this study only tested the reversal potential of one concentration each of PCC (40 U/mL) and rFVIIa (150 µg/kg), which may explain the lack of reversal of rivaroxaban-induced bleeding reported [46].

The concentrations of rivaroxaban used for the current study have been chosen to reflect maximum plasma concentrations expected after taking 20 mg once-daily rivaroxaban (range 175–400 ng/mL) or an extreme concentration to simulate overdose concentrations up to 1800 ng/mL. In healthy volunteers, there was a ceiling effect regarding plasma concentrations caused by limited absorption at doses above 50 mg [13]. In a previous study, the mean maximal plasma concentration of rivaroxaban was 316 ng/mL (range 185–532 ng/mL) after a single dose of 80 mg rivaroxaban in healthy volunteers [47]. Thus, the extreme concentrations tested in our study are unlikely to be achieved in clinical practice. Similarly, the concentrations of the haemostatic agents studied were chosen to reflect clinical practice, assuming a patient with a body weight of 70 kg [24,30,31].

All three haemostatic agents alone – in the absence of rivaroxaban – had a procoagulant effect on the parameters assessed. Moreover, some results indicated that these agents had the potential to overcompensate for the rivaroxaban-induced effect at lower concentrations of rivaroxaban. Thus, it will be important to measure the anticoagulation status of a patient before considering the use of a reversal agent in patients experiencing a severe or life-threatening bleeding event [13,48]. Moreover, to avoid increased risk of thromboembolic events, the half-life of the reversal agent should be considered. Some coagulation factors have a half-life of several hours and in patients in whom the rivaroxaban plasma concentration is already declining, their application may substantially increase thromboembolic risk. Clinical experience is required to evaluate the safety and efficacy profile of these haemostatic agents in rivaroxaban-anticoagulated patients.

In summary, all three haemostatic agents – rFVIIa, aPCC and PCC – were partially effective in reversing rivaroxaban-induced anticoagulation. In the clotting assays, rFVIIa and aPCC had a higher reversal potential than PCC, but reversal was not complete and reached a plateau. This limited reversal effect may partly be caused by the effect of the agent itself on the measurement (by accelerating the initiation phase of coagulation), suggesting that clotting times may not correspond to the clinical effects, and clotting assays may not precisely predict the reversal potential of haemostatic agents (in particular PCC). ETP measurements may be more predictive for assessing the reversal potential of PCC or aPCC, but not of rFVIIa. Data from this study indicate that PT, CT and TG assays cannot predict the exact dose of a reversal agent required to reverse anticoagulation given a specific plasma concentration of rivaroxaban. Therefore, more studies are required to establish the safety, efficacy and clinical utility of these agents for reversing the anticoagulant effect of rivaroxaban in clinical practice.

Conflict of Interest Statement

Elisabeth Perzborn is a former employee of and consultant for Bayer Pharma AG. Stefan Heitmeier, Volker Laux and Anja Buchmüller are employees of Bayer Pharma AG.

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References

[1] Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2012;141:e24S–43S.
[2] Weitz JI, Eikelboom JW, Samama MM. New antithrombotic drugs: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2012;141:e120S–51S.
[3] Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2012;141:e44S–88S.
[4] Eriksson BI, Borris LC, Friedman RJ, Haas S, Huisman MV, Kakkar AK, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. N Engl J Med 2008;358:2765–75.
[5] Kakkar AK, Brenner B, Dahl OE, Eriksson BI, Mouret P, Muntz J, et al. Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty: a double-blind, randomised controlled trial. Lancet 2008;372:31–9.
[6] Lassen MR, Ageno W, Borris LC, Lieberman JR, Rosencher N, Bandel TJ, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. N Engl J Med 2008;358:2776–86.
[7] Turpie AGG, Lassen MR, Davidson BL, Bauer KA, Gent M, Kwong LM, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial. Lancet 2009;373:1673–80.
[8] The EINSTEIN Investigators. Oral rivaroxaban for symptomatic venous thromboembolism. N Engl J Med 2010;363:2499–510.
[9] The EINSTEIN–PE Investigators. Oral rivaroxaban for the treatment of symptomatic pulmonary embolism. N Engl J Med 2012;366:1287–97.
[10] Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. N Engl J Med 2011;365:883–91.
[11] Mega JL, Braunwald E, Wiviott SD, Bassand JP, Bhatt DL, Bode C, et al. Rivaroxaban in patients with a recent acute coronary syndrome. N Engl J Med 2012;366:9–19.
[12] Holbrook A, Schulman S, Witt DM, Vandvik PO, Fish J, Kovacs MJ, et al. Evidence-based management of anticoagulant therapy: antithrombotic therapy and prevention of thrombosis: 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 2012;141:e152S–84S.
[13] Bayer Pharma AG. Xarelto® Summary of Product Characteristics. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000944/WC500057108.pdf; 2013.
[14] Boehringer Ingelheim International GmbH. Pradaxa® Summary of Product Characteristics. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000829/WC500041059.pdf; 2013.
[15] Bristol-Myers Squibb, Pfizer EEIG. Eliquis® Summary of Product Characteristics. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002148/WC500107728.pdf; 2013.
[16] Bristol-Myers Squibb. Coumadin Prescribing Information. Available at: http://packageinserts.bms.com/pi/pi_coumadin.pdf; 2011.
[17] Kubitza D, Becka M, Wensing G, Voith B, Zuehlsdorf M. Safety, pharmacodynamics, and pharmacokinetics of BAY 59–7939 – an oral, direct Factor Xa inhibitor – after multiple dosing in healthy male subjects. Eur J Clin Pharmacol 2005;61:873–80.
[18] Kubitza D, Becka M, Roth A, Mueck W. Dose-escalation study of the pharmacokinetics and pharmacodynamics of rivaroxaban in healthy elderly subjects. Curr Med Res Opin 2008;24:2757–65.
[19] Scharrer I. Recombinant Factor VIIa for patients with inhibitors to Factor VIII or IX or Factor VII deficiency. Haemophilia 1999;5:253–9.
[20] Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA. Prospective, randomised trial of two doses of rFVIIa (NovoSeven) in haemophilia patients with inhibitors undergoing surgery. Thromb Haemost 1998;80:773–8.
[21] Lusher JM, Shapiro SS, Palascak JE, Rao AV, Levine PH, Blatt PM. Efficacy of prothrombin-complex concentrates in hemophiliacs with antibodies to factor VIII: a multicenter therapeutic trial. N Engl J Med 1980;303:421–5.
[22] Sjamsoedin LJ, Heijnen L, Mauser-Bunschoten EP, van Geijlswijk JL, van Houwelingen H, van Asten P, et al. The effect of activated prothrombin-complex concentrate (FEIBA) on joint and muscle bleeding in patients with hemophilia A and antibodies to factor VIII. A double-blind clinical trial. N Engl J Med 1981;305:717–21.
[23] Negrier C, Goudemand J, Sultan Y, Bertrand M, Rothschild C, Lauroua P. Multicenter retrospective study on the utilization of FEIBA in France in patients with factor VIII and factor IX inhibitors. French FEIBA Study Group. Factor Eight Bypassing Activity. Thromb Haemost 1997;77:1113–9.
[24] CSL Behring GmbH. Beriplex P/N 250 IU Summary of Product Characteristics. Available at: http://www.medicines.org.uk/emc/medicine/21147/SPC/Beriplex+P+N+250+&+500+IU; 2013.
[25] CSL Behring GmbH. Kcentra Prescribing Information. Available at: http://labeling.cslbehring.com/PI/US/Kcentra/EN/Kcentra-Prescribing-Information.pdf; 2013.
[26] Eerenberg ES, Kamphuisen PW, Sijpkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. Circulation 2011;124:1573–9.
[27] Zhou W, Zorn M, Nawroth P, Butehorn U, Perzborn E, Heitmeier S, et al. Hemostatic therapy in experimental intracerebral hemorrhage associated with rivaroxaban. Stroke 2013;44:771–8.
[28] Perzborn E, Gruber A, Tinel H, Marzec UM, Buetehorn U, Buchmueller A, et al. Reversal of rivaroxaban anticoagulation by haemostatic agents in rats and primates. Thromb Haemost 2013;110:162–72.
[29] Mueck W, Lensing AWA, Agnelli G, Décousus H, Prandoni P, Misselwitz F. Rivaroxaban: population pharmacokinetic analyses in patients treated for acute deep-vein thrombosis and exposure simulations in patients with

atrial fibrillation treated for stroke prevention. Clin Pharmacokinet 2011;50:675–86.
[30] Immuno Ltd. FEIBA Summary of Product Characteristics. Available at: http://www.mhra.gov.uk/home/groups/l-unit1/documents/websiteresources/con014534.pdf; 2007.
[31] Novo Nordisk. NovoSeven® RT Coagulation Factor VIIa (recombinant) Prescribing Information. Available at: http://www.novosevenrt.com/pdfs/PI_novosevenrt.pdf; 2010.
[32] Gerotziafas GT, Elalamy I, Depasse F, Perzborn E, Samama MM. *In vitro* inhibition of thrombin generation, after tissue factor pathway activation, by the oral, direct Factor Xa inhibitor rivaroxaban. J Thromb Haemost 2007;5:886–8.
[33] Sørensen B, Spahn DR, Innerhofer P, Spannagl M, Rossaint R. Clinical review: Prothrombin complex concentrates–evaluation of safety and thrombogenicity. Crit Care 2011;15:201.
[34] Samama MM, Martinoli JL, Le Flem L, Guinet C, Plu-Bureau G, Depasse F, et al. Assessment of laboratory assays to measure rivaroxaban – an oral, direct Factor Xa inhibitor. Thromb Haemost 2010;103:815–25.
[35] Molenaar PJ, Dinkelaar J, Leyte A. Measuring rivaroxaban in a clinical laboratory setting, using common coagulation assays, Xa inhibition and thrombin generation. Clin Chem Lab Med 2012;50:1799–807.
[36] Brummel KE, Paradis SG, Butenas S, Mann KG. Thrombin functions during tissue factor-induced blood coagulation. Blood 2002;100:148–52.
[37] Körber MK, Langer E, Ziemer S, Perzborn E, Gericke C, von Heymann C. Measurement and reversal of prophylactic and therapeutic peak levels of rivaroxaban: an *in vitro* study. Clin Appl Thromb Hemost 2013. http://dx.doi.org/10.1177/1076029613494468.
[38] Dinkelaar J, Molenaar PJ, Ninivaggi M, de Laat B, Brinkman HJ, Leyte A. *In vitro* assessment, using thrombin generation, of the applicability of prothrombin complex concentrate as an antidote for Rivaroxaban. J Thromb Haemost 2013;11:1111–8.
[39] Fukuda T, Honda Y, Kamisato C, Morishima Y, Shibano T. Reversal of anticoagulant effects of edoxaban, an oral, direct factor Xa inhibitor, with haemostatic agents. Thromb Haemost 2012;107:253–9.
[40] Escolar G, Fernandez-Gallego V, Arellano-Rodrigo E, Roquer J, Reverter JC, Sanz VV, et al. Reversal of apixaban induced alterations in hemostasis by different coagulation factor concentrates: significance of studies *in vitro* with circulating human blood. PLoS One 2013;8:e78696.
[41] Hemker HC, Giesen PL, Ramjee M, Wagenvoord R, Beguin S. The thrombogram: monitoring thrombin generation in platelet-rich plasma. Thromb Haemost 2000;83:589–91.
[42] Gabriel DA, Li X, Monroe III DM, Roberts HR. Recombinant human factor VIIa (rFVIIa) can activate factor FIX on activated platelets. J Thromb Haemost 2004;2:1816–22.
[43] Desmurs-Clavel H, Huchon C, Chatard B, Negrier C, Dargaud Y. Reversal of the inhibitory effect of fondaparinux on thrombin generation by rFVIIa, aPCC and PCC. Thromb Res 2009;123:796–8.
[44] Marlu R, Hodaj E, Paris A, Albaladejo P, Crackowski JL, Pernod G. Effect of non-specific reversal agents on anticoagulant activity of dabigatran and rivaroxaban. A randomised crossover *ex vivo* study in healthy volunteers. Thromb Haemost 2012;108:217–24.
[45] Brummel-Ziedins KE, Orfeo T, Gissel M, Mann KG, Rosendaal FR. Factor Xa generation by computational modeling: an additional discriminator to thrombin generation evaluation. PLoS One 2012;7:e29178.
[46] Godier A, Miclot A, Le Bonniec B, Durand M, Fischer AM, Emmerich J, et al. Evaluation of prothrombin complex concentrate and recombinant activated Factor VII to reverse rivaroxaban in a rabbit model. Anesthesiology 2012;116:94–102.
[47] Kubitza D, Becka M, Voith B, Zuehlsdorf M, Wensing G. Safety, pharmacodynamics, and pharmacokinetics of single doses of BAY 59–7939, an oral, direct Factor Xa inhibitor. Clin Pharmacol Ther 2005;78:412–21.
[48] Turpie AGG, Kreutz R, Llau J, Norrving B, Haas S. Management consensus guidance for the use of rivaroxaban - an oral, direct Factor Xa inhibitor. Thromb Haemost 2012;108:876–86.